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CASE REPORT

Ciprofloxacin-Resistant *Aeromonas hydrophila* Cellulitis following Leech Therapy

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We report a case of surgical site infection with ciprofloxacin-resistant *Aeromonas hydrophila* following leech therapy. Antimicrobial and genetic analyses of leech and patient isolates demonstrated that the resistant isolates originated from the leech gut microbiota. These data suggest that ciprofloxacin monotherapy as a prophylaxis regimen prior to leech therapy may not be effective in preventing infection.

CASE REPORT

A 9-year-old female with otocephalic mandibular syndrome underwent bilateral mandibular osteotomy with placement of a mandibular distraction and planned free-tissue transfer reconstruction with a supraclavicular island pedicle flap from the left side of the neck. Perioperatively, the patient was treated with prophylactic cefazolin. Duskiness suggestive of venous congestion at the distal portion of the flap was observed 48 h postoperatively. Leeches were applied following ciprofloxacin prophylaxis (200 mg administered by gastrostomy tube twice a day) in accordance with standard hospital practice; however, leech therapy did not correct the congested state and the patient was discharged and scheduled for debridement and removal of the distal portion of the flap while receiving oral cephalexin for prophylaxis.

While awaiting surgery, the patient presented to the emergency department with purulent drainage from the mandibular hardware and dehiscence of the wound surrounding the mandibular distraction device. She was admitted and received vancomycin (360 mg intravenously [i.v.] every 8 h [q8h]) and local wound care. The wound was debrided, and the left supraclavicular flap was replaced; samples of necrotic tissue from the flap and around the distraction arm were sent to the microbiology laboratory for anaerobic and aerobic bacterial cultures. Anaerobic cultures grew *Prevotella* sp. and *Bacteroides fragilis*, while aerobic cultures grew *Morganella morganii* and *Aeromonas hydrophila*. Anaerobic cultures were grown on brucella blood agar, phenyl ethyl alcohol, and laked kanamycin vancomycin agars (Anaerobe Systems, Morgan Hill, CA). Identification to the species level was done on the basis of Gram stain results in combination with special-potency antibiotic disk patterns, bile esculin, colony morphology, spot indole, and catalase tests. Aerobic cultures were grown on sheep blood agar, chocolate agar, and MacConkey agar plates (BBL, Sparks, MD); identification to the species level was done by using API 20NE (BioMérieux, Durham, NC) and 16S rRNA gene sequence analysis (1). Vancomycin was discontinued, and the patient was placed on cefepime (1.15 mg i.v. q8h), clindamycin (200 mg i.v. q8h) and metronidazole (230 mg i.v. q8h) pending susceptibility testing results.

Antimicrobial susceptibility testing of the *M. morganii* and *A. hydrophila* isolates was performed by the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method (2) on panels prepared in-house. *M. morganii* was susceptible to cefepime, gentamicin, and piperacillin-tazobactam, with MICs of ≤0.5, 1.0, and ≤8 μg/ml, respectively. Similarly, *A. hydrophila* isolates from the patient's face (AH1) and from the distraction arm (AH2) were susceptible to cefepime, gentamicin, ceftriaxone, and piperacillin-tazobactam, with MICs of ≤0.5, ≤0.5, ≤0.5, and ≤8 μg/ml, respectively (Table 1). Both the AH1 and AH2 isolates were resistant to ampicillin-sulbactam and cefazolin (>32 μg/ml). Most notably, *M. morganii* had a ciprofloxacin MIC indicating intermediate resistance (2 μg/ml) and AH1 and AH2 had a ciprofloxacin MIC indicating resistance (4 μg/ml) (Table 1).

Upon the receipt of antimicrobial susceptibility testing results, cefepime and clindamycin treatment was discontinued and the patient was treated with piperacillin-tazobactam (2.25 g i.v. q8h) and metronidazole treatment (230 mg i.v. q8h) was continued for 3 weeks. Two additional surgeries were performed on hospitalization days 15 and 24 to revise the supraclavicular island pedicle flap, for tissue debridement, and to remove to the distraction arms. The patient was discharged from the hospital on day 23. After discharge, the patient was followed up in the outpatient clinic. Four months following the original surgery, her wounds appeared to be healing well.

We sought to determine whether the ciprofloxacin resistance of the patient's *Aeromonas* isolate was due to antibiotic selection for resistant isolates in the patient or whether the leech gut microbiota itself harbored the resistant isolates. Leeches purchased from Leeches USA from the same lot used on the patient and water from the tank used to house the leeches were collected from the hospital pharmacy. Leeches were treated as previously described to culture the gut microbiota (3). Briefly, tubes of sterile whole human blood collected in EDTA were sealed with Parafilm; small holes were made in the Parafilm, and the leeches were allowed to feed until release. The anterior and posterior ends of the leech were tied, and the leech was immersed in ethanol for 10 s for sterilization. The

Received 6 December 2012 **Returned for modification** 6 January 2013
Accepted 19 January 2013
Published ahead of print 30 January 2013
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doi:10.1128/JCM.03217-12

TABLE 1 MICs for the *A. hydrophila* isolates used in this study

Drug	MIC[a] (μg/ml) for *A. hydrophila* isolate (resistance phenotype)			
	AH1	AH2	AH3	AH4
Ampicillin	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)
Ampicillin-sulbactam	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)
Cefazolin	≥32 (R)	32 (R)	≥32 (R)	≥32 (R)
Cefepime	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)
Gentamicin	≤0.5 (S)	≤0.5 (S)	1 (S)	≤0.5 (S)
Piperacillin-tazobactam	≤8/2 (S)	≤8/2 (S)	≤8/2 (S)	≤8/2 (S)
Ciprofloxacin	4 (R)	4 (R)	1 (S)	≤0.015 (S)
Levofloxacin	8 (R)	4 (I)	≤2 (S)	≤2 (S)
Minocycline	1 (—)[b]	≤0.5 (—)[b]	1 (—)[b]	≤0.5 (—)[b]
Trimethoprim-sulfamethoxazole	≤1/20 (S)	≤1/20 (S)	≥4/80 (R)	≤1/20 (S)

[a] *A. hydrophila* isolates were obtained from the patient's face (AH1), the distraction arm (AH2), the blood tube after leech feeding (AH3), and the leech tank water (AH4). Phenotypes: R, resistant; S, susceptible; I, intermediate.
[b] —, No interpretive criteria.

midgut was dissected and plated on 5% sheep blood agar and chocolate agar plates (BBL, Sparks, MD). Plates were incubated for 24 h in a 5% CO_2 environment at 35°C. Midgut dissection cultures were overgrown with *Proteus*; however, culture from the blood tube after leech feeding and culture of the tank water revealed *A. hydrophila* isolates (AH3 and AH4, respectively). *A. hydrophila* identification was confirmed by 16S rRNA gene sequence analysis as described elsewhere (1), with >99.0% identity to the reference sequence. The 16S rRNA gene sequences of AH1, AH2, and AH3 were identical, suggesting that these isolates were clonal in nature (not shown). Susceptibility testing of the patient and leech isolates was performed by the CLSI broth microdilution reference method (2). Ciprofloxacin MICs of 1 and ≤0.015 μg/ml were observed for isolates from the blood tube (AH3) and tank (AH4), respectively. The mechanism of fluoroquinolone resistance was investigated by sequence analysis of the *gyrA* genes. *gyrA* was amplified from total bacterial genomic DNA by using forward primer *gyrA*-F (5′-ATGAGCGATCTGGCCAGAGA-3′) and reverse primer *gyrA*-R (5′-CAATACCGGAGGAGCCGTT-3′). The final concentrations of reagents in a 25-μl reaction mixture were as follows: 1× AmpliTaq Gold PCR Master Mix (Life technologies, Grand Island, NY), 1 μM each primer, and 50 to 200 ng template DNA. Cycling parameters of 95°C for 5 min; 35 cycles of 95°C for 1 min, 65°C for 1 min, and 72°C for 2 min; and extension for 7 min at 72°C were used. Sequence comparison of all of the isolates revealed a serine-to-isoleucine point mutation at codon 83 of the *gyrA* gene in the AH1, AH2, and AH3 isolates, whereas AH4 harbored the wild-type allele (data not shown). No other mutations in AH1, AH2, or AH3 were detected. These data suggest that AH1 and AH2 acquired the resistance mutation prior to exposure to ciprofloxacin in the patient and harbored the same quinolone resistance mutation as AH3, which was isolated from the leech.

Medicinal leeches (*Hirudo medicinalis*), used primarily in plastic and reconstructive surgeries to prevent venous congestion, maintain *A. hydrophila*, which is essential for the digestion of host erythrocytes, as an obligatory endosymbiont (4, 5). Upon attachment to the skin, *H. medicinalis* bites the host and injects saliva that contains numerous anticoagulants and vasodilators to promote continuous blood supply during the leech blood meal (5, 6). In response to the widespread use of medicinal leeches, *H. medicinalis* received official approval as a medical device from the Food and Drug Administration in 2004.

A. hydrophila infection subsequent to leech therapy has been well documented (7–10), accounting for 88% of leech therapy infectious complications (10). Because of the high incidence of infection associated with the application of leeches, antimicrobial prophylaxis of patients prior to leech therapy is recommended, although the class and dose of antibiotic are not well standardized (9). A 2004 survey of U.S. plastic surgery clinics revealed that 30% used amoxicillin-clavulanate, 22% used ciprofloxacin, and 10% used a cephalosporin or metronidazole as prophylaxis prior to leech therapy (9). Current evidence suggests that the most effective prophylactic regimen includes a fluoroquinolone (10).

Resistance to fluoroquinolones in *Aeromonas* species, via the plasmid-mediated quinolone resistance determinant *qnrS2*, has been reported in a number of environmental isolates from lakes and natural water sources (11–14). The Qnr protein contains a pentapeptide repeat region that is predicted to act as a DNA analogue to compete for fluoroquinolone-mediated DNA gyrase inhibition (15). While the presence of this plasmid is prevalent in many members of the family *Enterobacteriaceae* (14) quinolone resistance does not appear to be widespread among environmental aeromonads (11–14). Historical surveys of *A. hydrophila* isolated from medicinal leeches demonstrated consistent susceptibility to ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole (16). However, infection by ciprofloxacin-resistant *A. hydrophila* concurrent with leech therapy was recently described by others (8, 17). In both cases, ciprofloxacin resistance was found only in *Aeromonas* isolated from the patient specimens. Wang and colleagues found concurrent ciprofloxacin and trimethoprim-sulfamethoxazole resistance in *A. hydrophila* isolates following leech therapy; similarly, we noted trimethoprim-sulfamethoxazole resistance in the AH3 isolate. Consistent with our findings of *Aeromonas* isolated from tank water and the exterior surface of the leech (data not shown), Wang and colleagues did not find any resistant isolates in the water or on the tank surface.

It has recently been demonstrated that ciprofloxacin feeding eliminates *Aeromonas* spp. from the leech midgut while maintaining the leech's ability to take a blood meal (18). However, it is unclear whether such treatment would reduce the number of nosocomial infections due to leech therapy or promote resistance in these organisms, in particular if *Aeromonas* harbored in the leech gut expressed reduced susceptibility to ciprofloxacin, such as in the isolates investigated herein.

In light of these data, consideration of additional antibiotic prophylaxis may be warranted when medicinal leeches are used, such as a combination of fluoroquinolone and trimethoprim-sulfamethoxazole or a tetracycline. However, tetracyclines are not a viable alternative for children ≤8 years old. Of note, isolate AH3 was found to be resistant to trimethoprim-sulfamethoxazole. Treating clinicians should also be cognizant that *Aeromonas* species may possess multiple inducible β-lactamases and that resistance may emerge during the course of therapy with a β-lactam by the promotion of a previously unexpressed β-lactamase (19, 20). Alternatively, suppliers of FDA-approved medicinal leeches could be required to adhere to susceptibility testing.

ACKNOWLEDGMENT

We thank Meg Kanatani for her help collecting leeches and tank water for analysis.

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